

Video Article

Dissection of 6.5 dpc Mouse Embryos

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Abstract

Analysis of gene expression patterns during early stages of mammalian embryonic development can provide important clues about gene function, cell-cell interaction and signaling mechanisms that guide embryonic patterning. However, dissection of the mouse embryo from the decidua shortly after implantation can be a challenging procedure, and detailed step-by-step documentation of this process is lacking.

Here we demonstrate how post-implantation (6.5 dpc) embryos are isolated by first dissecting the uterus of a pregnant mouse (detection of the vaginal plug was designated day 0.5 post coitum) and subsequently dissecting the embryo from maternal decidua. The dissection of Reichert's membrane is described as well as the removal of the ectoplacental cone.

Protocol

Dissection of reproductive organs from a female mouse

1. Pregnant animals are euthanized by CO₂ asphyxiation using compressed gas.
2. Lay the animal supine on absorbent pad and soak it in 70% ethanol to reduce the risk of contaminating the dissection with mouse hair.
3. Pinch the skin and make a small lateral incision at the midline with regular surgical scissors. Hold the skin firmly above and below the incision and pull the skin apart toward the head and tail to expose the abdomen.
4. Grasp the peritoneum with forceps and cut to expose the abdominal cavity.
5. Locate the reproductive organs in the dorsal region of the body cavity: two uterine horns, the oviduct and the ovaries.

Primitive Streak-stage (6.5 dpc) Dissection

1. Remove the uterine horn by grasping the uterus below the oviduct and cut it free along the mesometrium. Place dissected uterus in ice cold PBS.



Figure 1

2. Separate each embryo by cutting between implantation sites along uterine horn.

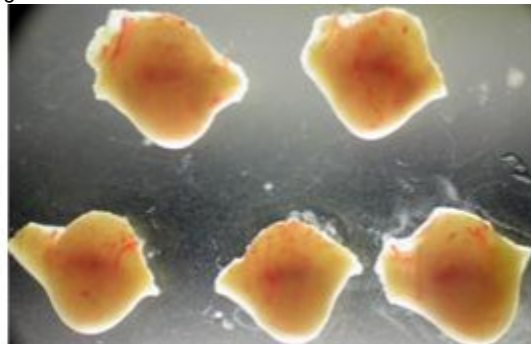


Figure 2

3. Grasp the muscular uterine lining by sliding watchmaker's forceps between the surrounding muscle layer and enveloped decidua tissue. Peel back the muscle layer, exposing the decidua.

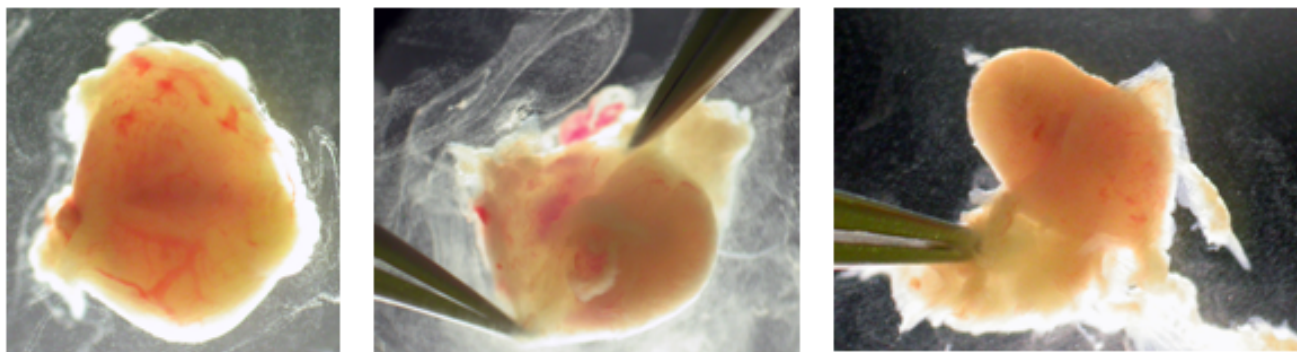


Figure 3

4. Clip off a portion of the exposed decidua at the apex (approximately 1/5 of the decidua tissue) which will expose the midventral or distal tip of the enclosed embryo.

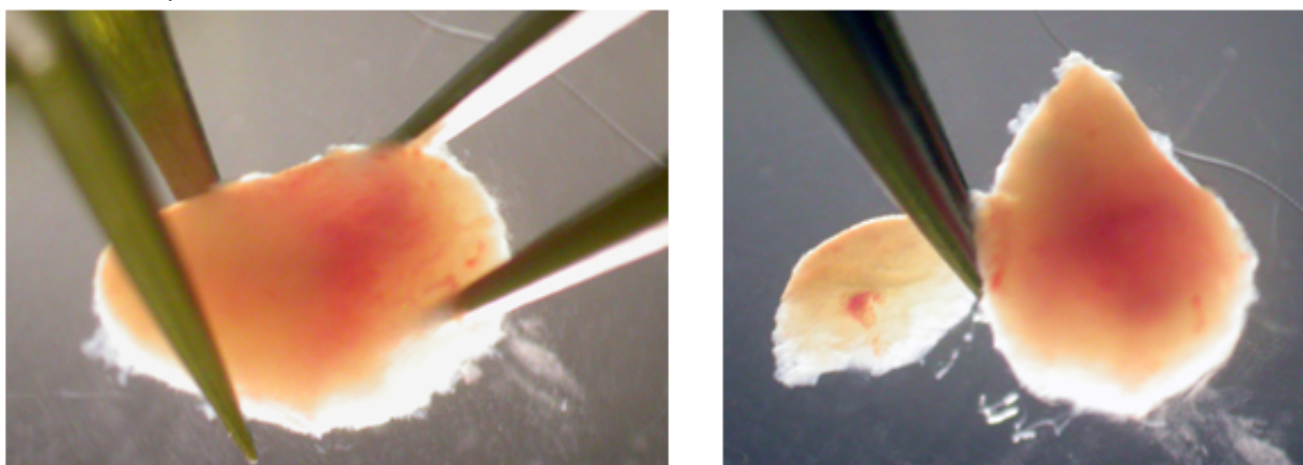


Figure 4

5. The embryo can be shelled out using the tips of forceps. Pierce the decidua with forceps surrounding the embryo and open forceps to tear decidua apart.

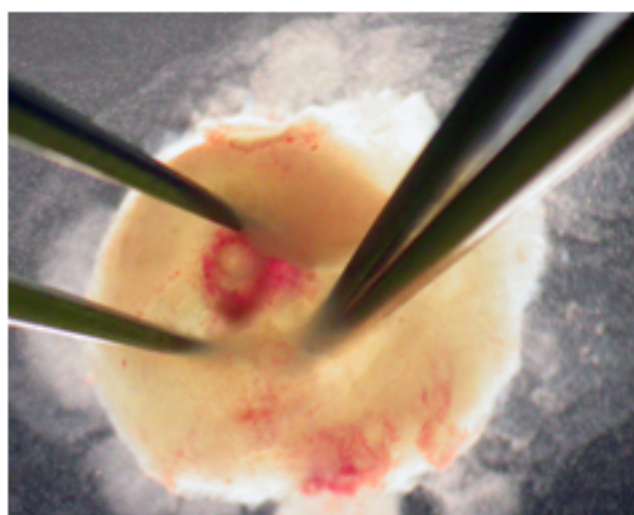


Figure 5

6. Once embryo is removed, Reichart's membrane may still be attached as well as the ectoplacental cone (trophoblast). The embryo can be dissected further by careful dissection.

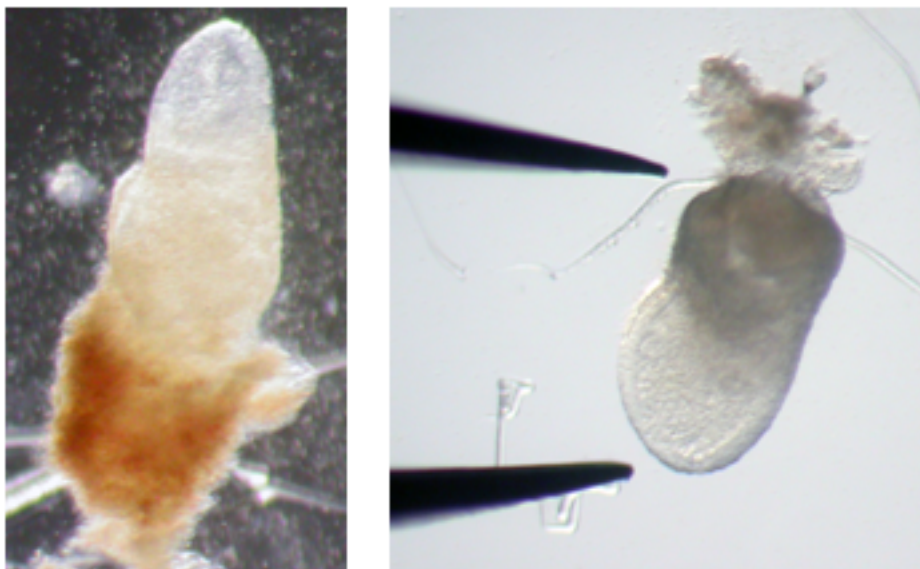


Figure 6

Discussion

Postimplantation in the mouse occurs between 4.5 dpc and birth. This dissection method can be applied, essentially as described, to isolate earlier or later stage embryos at 5.5-8.5 dpc.

References

1. Nagy, A. et. al. 2003. Manipulating the mouse embryo: A laboratory manual third edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.